

Patent Claims

1. Method for identification of a pathogenic organism from a predetermined group of pathogens, comprising
 - a) at least partially purifying nucleic acid from a clinical sample,
 - 5 b) subjecting at least a first aliquot of said clinical specimen to at least one amplification and detection reaction in one reaction vessel comprising
 - ba) an amplification step using at least a first set of amplification primers capable of amplifying a pre-selected nucleic acid sequence region from several or all members of said predetermined group of pathogens,
 - 10 bb) a detection step using at least 2, 3 or multiple hybridization reagents, said reagents together being capable of specifically detecting a pre-selected nucleic acid sequence region from all members of said group of pathogens, said detection step bb) comprising steps
 - 15 bba) monitoring hybridization of each of said hybridization reagents at a pre-selected temperature, said hybridization being indicative for at least the genus of said pathogen present in the sample, and
 - bbb) monitoring temperature dependence of hybridization, said temperature dependence being indicative for at least the species of said pathogen, determining whether said amplification and detection reaction is
20 indicative for the presence of a specific member of said pre-selected group of pathogens.
2. Method according to claim 1, wherein a first and a second aliquot each are subjected to an amplification and detection reaction independently from each other in two different reaction vessels,
- 25 3. Method according to claim 2, wherein a first, a second and a third aliquot each are subjected to an amplification and detection reaction independently from each other in two different reaction vessels.

4. Method according to claims 1-3, wherein an additional hybridization reagent is used for the detection of an internal control.
5. Method according to claim 2, wherein gram positive pathogenic organisms are exclusively identified in one amplification and detection reaction and gram negative pathogenic organisms are exclusively identified in another amplification and detection reaction.
6. Method according to claim 3 and 5, wherein fungal pathogens are exclusively identified in the third amplification and detection reaction.
7. Method according to claims 2-6, wherein each amplification step is performed with the same thermocycling profile.
8. Composition comprising at least a first set of amplification primers and at least two, three or multiple hybridization reagents, characterized in that
 - said at least first set of amplification primer is capable of amplifying a pre-selected nucleic acid sequence region from several or all members of a predetermined group of pathogens, and
 - said at least 2, 3 or multiple hybridization reagents together are being capable of specifically detecting a pre-selected nucleic acid sequence region from all members of a predetermined group of pathogens, wherein
 - hybridization of each of said hybridization reagents at a pre-selected temperature is indicative for at least the genus of a pathogen present in the sample, and
 - the temperature dependence of said hybridization is indicative for at least the species of said pathogen.
9. Kit comprising at least a first set of amplification primers and at least two, three or multiple hybridization reagents, characterized in that

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- said at least first set of amplification primer is capable of amplifying a pre-selected nucleic acid sequence region from several or all members of a predetermined group of pathogens, and
- 5 - said at least 2, 3 or multiple hybridization reagents together are being capable of specifically detecting a pre-selected nucleic acid sequence region from all members of a predetermined group of pathogens, wherein
 - 10 - hybridization of each of said hybridization reagents at a pre-selected temperature is indicative for at least the genus of a pathogen present in the sample, and
 - the temperature dependence of said hybridization is indicative for at least the species of said pathogen.